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# Histological Effects and Bioaccumulation Potential of Coal Particulate-Bound Phenanthrene in the Fathead Minnow 'Pimephales promelas'

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# HISTOLOGICAL EFFECTS AND BIOACCUMULATION POTENTIAL OF COAL PARTICULATE-BOUND PHENANTHRENE IN THE FATHEAD MINNOW *PIMEPHALES PROMELAS*

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*ABSTRACT*

*Fathead minnows* Pimephales promelas *were exposed to phenanthrene adsorbed to < 125 μm coal particles in a 14-day flow-through exposure to determine changes in histology and bioaccumulation. Based on light microscopic examination, coal particulates caused no obvious damage to external surfaces such as gills or skin and particulates were not observed to have accumulated in internal tissues. However, coal particles were readily ingested and this was followed by extensive mucous secretion by the gut mucosa. In addition, electron micrographs of the gut showed mucous globules in coal-exposed epithelium but no erosion of microvilli. Phenanthrene bioaccumulation was compared in fish exposed to dissolved aqueous phenanthrene and fish exposed to dissolved aqueous phenanthrene plus coal particulate-bound phenanthrene. Enhanced phenanthrene uptake was not measured in coal-exposed fish and bioconcentration factors were 1000–4000 during the 14-day exposure period. Elimination of coal from the gut and phenanthrene from the tissues was rapid; both were undetectable after a 24-h depuration period.*

## INTRODUCTION

Particulate emissions during the mining, transport, storage and combustion of coal are of concern during this period of increased emphasis on coal-energy technology. For example, several field studies have demonstrated adverse effects on aquatic organisms from particulates associated with coal-mining operations. These effects included a reduction in abundance, diversity, growth and spawning of fish and

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macroinvertebrates in contaminated rivers attributable to coal particulates (Williams & Harcup, 1974; Scullion & Edwards, 1980) or to precipitated iron hydroxide particulates (Greenfield & Ireland, 1978; Letterman & Mitsch, 1978).

Laboratory studies with fish exposed to particulates have used survival, growth and behavioural assays as effect endpoints. The most commonly studied particulate types have been sediments, with lethality usually attained only at very high levels (1000–100,000 mg/litre) of suspended sediments and clays (Cordone & Kelley, 1961; Auld & Schubel, 1978; Sprague & Logan, 1979). Coal particulates were not toxic to rainbow trout at 200 mg/litre but growth was depressed (Herbert & Richards, 1963). Also, inhibited growth was observed in coho salmon and fathead minnows exposed to less than 2 mg/litre ferric hydroxide suspensions (Smith *et al.*, 1973; Smith & Sykora, 1976). The behavioural responses of particulate-exposed fishes have been measured using cough rate (Hughes, 1975) and avoidance assays (Lawrence & Scherer, 1974). Hughes found that coal dust caused a twofold increase in rainbow trout cough rate at concentrations of 120–600 mg/litre. Both laboratory and field studies to determine the effects of suspended particulates on freshwater organisms have been reviewed by Sorensen *et al.* (1977).

The limited histological and morphological observations of particulate-exposed fishes have focused on gill morphology. Herbert & Merkens (1961) observed thickening and fusion of gill lamellae in rainbow trout exposed to high concentrations of kaolin or diatomaceous earth. However, not all individuals were affected; some fish had normal gills even after an 8-month exposure period. In a coal ash seepage area Coutant *et al.* (1978) observed accumulation of ferric hydroxide on the gills of caged channel catfish. Clogging of opercular cavities was also noted in several species of fish exposed to sediment concentrations of 100,000 to 225,000 mg/litre (Wallen, 1951).

The effect of particulates on bioaccumulation has been examined primarily with respect to the benthic environment. In experiments with petroleum and PCB-contaminated sediments, polychaetes and bivalves accumulated these compounds more readily after release into the surrounding water than while bound in sediments (Courtney & Langston, 1978; Roesijadi *et al.*, 1978). Southworth *et al.* (1979) estimated the relative importance of water versus sediment uptake of acridine by fathead minnows and estimated that 98 % was contributed by aqueous acridine and only 1 % by sediment-adsorbed acridine. In contrast, when suspended particulates (alumina or silica) were used, PCBs were apparently accumulated from these particles in bivalves (Langston, 1978) and fish (Zitko, 1974). However, in the latter two studies the water was not analysed and suitable controls were not run.

Since 1975, northern Great Plains coal has been received, stored and shipped in the Duluth-Superior harbour, located at the western tip of Lake Superior. It has been estimated that 20 metric tons of coal particulates are deposited annually in the harbour contributed by wind action on storage piles, grooming of coal piles and ship loading operations (Sydor & Stortz, 1980). Therefore physical, chemical and

biological studies are being done to assess possible regional environmental hazards. In our preliminary biological studies, laboratory exposure of fathead minnows to high coal particulate concentrations (25,000 mg/litre) resulted in 100% mortality after 96 h (Carlson *et al.*, 1979). However, field measurements of coal particulates in channels near the loading dock showed levels at approximately 1 mg/litre (Sydor & Stortz, 1980). We therefore describe in this paper physiological effects of exposure to low concentrations (10 mg/litre) of coal particulates using the fathead minnow *Pimephales promelas*. Coal particles were coated with phenanthrene, a three-ring polycyclic aromatic hydrocarbon, so that, in addition to particulate-induced histological changes, particulate-enhanced bioaccumulation could be evaluated.

## MATERIALS AND METHODS

### *Preparation of coal slurry*

Sub-bituminous coal mined in Decker, Montana, USA, was shipped by rail to Superior, Wisconsin, USA. In October 1975, samples were collected from several railway cars, yielding a 30 kg total sample weight. This sample was mixed in a large fibre barrel and subsequently ground in a Wiley mill. The sieved coal was stored in sealed brown glass bottles until use.

One thousand millilitres of $<125$-$\mu$m sieved coal were added to 12 litres of purified water. This water had been prepared by filtration through 0·45-$\mu$m HA Millipore® filters followed by elution through a 60 × 7-mm column packed with C-18 Corasil®. The coal–water mixture was shaken and allowed to settle for 20 h. The resulting supernatant slurry was then used to prepare coal particulates with adsorbed phenanthrene. Ten triple-layer dialysis bags (12,000 + molecular weight) containing phenanthrene (Aldrich Chemical Co.) were added to the coal slurry. Each triple layer dialysis bag was prepared by reinforcing a central 1·6 cm diameter bag containing phenanthrene with two additional bags, 1·8 cm and 2·7 cm in diameter. The coal slurry and phenanthrene bags were placed in a stainless steel vessel under 10 psi nitrogen and shaken on a mechanical shaker for seven weeks. Periodically, the dialysis bags were checked for ruptures and replaced as required. The final coal slurry had a particulate concentration of 8760 mg/litre and a phenanthrene concentration of 160 mg per kilogramme of coal.

### *Flow-through system*

Two 45-litre glass aquaria were used for control and coal-treatment tanks. Filtered Lake Superior water was pumped into each tank at a rate of approximately 180 ml/min, maintaining a temperature of $22·5 \pm 0·4$ °C. Filtered water was generated by passing Lake Superior water through a 25 $\mu$m filter cartridge (Cuno, Aqua-Pure®, model AP110), a 1 $\mu$m cartridge (Gelman, Perflow 200, model 12622) and then a pressure gauge before entering a volume-regulated stainless steel

headbox. A Masterflex peristaltic pump (model 7015, Cole-Parmer) was used to deliver the filtered lake water from the headbox to the tanks. To facilitate efficient particulate draining under flow-through conditions, a glass sleeve (17 mm inside diameter) notched at the base, was fitted over each glass standpipe (8 mm inside diameter) causing the effluent to drain near the bottom of the tank. An airstone was present in each tank to keep the particles in suspension and to maintain sufficient dissolved gas concentrations.

When the phenanthrene-containing coal particles were diluted with Lake Superior water to simulate bioassay exposure conditions, approximately 0·08 μg/litre phenanthrene was measured in the water and approximately 1 μg/litre phenanthrene in suspended coal particles. Therefore the control tank was set up with approximately 0·08 μg/litre dissolved phenanthrene but lacking coal particulates. For the control tank a stock solution, containing 278 μg/litre dissolved aqueous phenanthrene, was diluted with filtered Lake Superior water in a 20-litre glass carboy. This dilute stock solution was freshly prepared each day and pumped at a rate of 10 ml/min into the control tank using a Gilson (Minipuls II) peristaltic pump fitted with 4 mm inside diameter silicone tubing. For the coal tank an aliquot of the stock coal slurry was diluted each day with filtered Lake Superior water in a 48-litre glass carboy. This coal particulate suspension, which was stirred continually with an overhead stirring motor to retard settling in the reservoir, was pumped at a rate of 20 ml/min from the reservoir to the treatment tank with a Gilson peristaltic pump (Minipuls II) fitted with several 2 mm inside diameter Tygon tubes. The resulting particulate concentration in the tank was approximately 10 mg/litre.

To initiate the experiment, 1100 fathead minnows were divided between the two tanks. The cohort was 6–9 weeks old and had a total length range of 12–35 mm. They were held under a 16:8 h (light:dark) photoperiod and fed No. 1 pellets (Glencoe Mills, Inc.) and live brine shrimp nauplii. The tanks were scrubbed and siphoned daily. Fish were monitored for 14 days during the coal/phenanthrene exposure and for 3 days after the coal and phenanthrene inputs were stopped. Both tanks received only Lake Superior water during this 3-day clearance period and the coal tank cleared visibly during the first 4 h.

*Static exposure*

A static short-term experiment was also conducted to monitor initial histological changes in fish exposed to coal. Several 10-week old minnows were placed into a 45-litre aquarium with a coal concentration of 20 mg/litre in filtered Lake Superior water. Two fish were collected hourly over a 7-h period.

*Histology*

During the flow-through exposure three fish per tank per day were collected for light microscopic examination on days 3 and 14 from the control tank and on days 1, 2, 3, 7, 14, 15, 16 and 17 from the coal tank. Two fish held in Lake Superior water but

with no added phenanthrene were also sampled for comparison with the phenanthrene-exposed controls. During the static exposure two fish were collected hourly over a 7-h period. All fish were immediately fixed in 10% neutral buffered formalin. They were then dehydrated in ethanol, infiltrated with JB-4 plastic embedding medium (Polysciences, Inc.) and sectioned at 3 μm on a DuPont/Sorvall JB-4 microtome. The sections were stained with methylene blue-azure II-basic fuchsin (Humphrey & Pittman, 1974).

For electron microscopic examination two fish from each tank were collected on day 14 of the flow-through exposure. Small pieces of gut were immediately fixed in Karnovsky's paraformaldehyde—glutaraldehyde fixative for 2 h at 4 °C. The tissues were rinsed in 0·2 M cacodylate buffer, cut into 1 mm cubes and post-fixed in 1% buffered $OsO_4$ for 1 h at 20 °C. After dehydration in ethanol and propylene oxide, the tissues were embedded in an epoxy resin (Epon 812-Araldite 502, Polysciences, Inc.), sectioned at 700 Å on a Sorvall Porter-Blum MT2-B ultramicrotome, and stained with lead citrate (Reynolds, 1963). Sections of the gut were observed over a magnification range of 3300 to 33,000 times in a JEOL JEM-100C electron microscope.

*Water analyses*

Particulate concentrations for each tank were measured twice daily in the coal tank and daily in the control tank. Each water sample (1–4 litres) was filtered through a tared GF/F microfibre filter. The filters were dried at 105 °C for 16–24 h before reweighing. The weighing error showed a consistent 3% variability among replicates.

Turbidity measurements were made each day with a Hach Model 2100A Turbidimeter. A sample collected from the coal exposure tank before fish were introduced was analysed in quadruplicate using a HIAC PC-320 Auto Particle Analyzer to determine particle size distribution. Water chemistry characteristics of three incoming Lake Superior water samples collected during the experiment were: pH = 7·2–7·4, alkalinity = 43·8 ± 0·3 mg/litre ($CaCO_3$, mean ± SD) and hardness = 47·5 ± 1·7 mg/litre ($CaCO_3$, mean ± SD).

The dissolved phenanthrene concentration in each tank was determined once a day by a high pressure liquid chromatographic (HPLC) technique. The HPLC system (Waters Associates) consisted of two M-6000 pumps, a Model 660 programmer, a U6K injector with a 17 ml sample loop and a Model 440 UV detector (254 nm). The analytical column was a Perkin Elmer C-18 10 μm reversed-phase column. The analysis proceeded by direct HPLC injection of a 12 ml sample of tank water which had been centrifuged at 1200 *g* for 30 min. A flow rate of 1·5 ml/min of 20% $CH_3CN$ in water was maintained and when a steady baseline was attained a programme of 20% to 100% acetonitrile in water was run over a 10-min period.

The phenanthrene content of the suspended solids from the coal exposure tank was measured by filtering 2–6 litres of tank water through 1 or 2 GF/F filters. The

filters were placed into a micro-Soxhlet apparatus and extracted for 5 h with 12 ml of methylene chloride. One microlitre of the methylene chloride extract was injected into a Tracor 550 gas chromatograph (GC) equipped with a 14 mm glass capillary column (OV-101) and a flame ionisation detector. A temperature programme of 70 to 190 °C at 39 °C/min was used.

*Fish analysis—Phenanthrene bioaccumulation*

Fish samples containing 15–50 fish were removed daily (day 1–day 17) from each tank during the flow-through exposure. In addition, on day 14, thirty fish from each tank were gutted to measure the contribution of ingested coal to the phenanthrene concentration of whole fish extracts. Samples were frozen at −20 °C in 4-dram glass vials until extraction. After thawing, the samples were weighed and the fish ground in a Waring blender with 70–100 g sodium sulphate. Each fish sample was Soxhlet extracted with 170 ml of methylene chloride for 9 h and the extract concentrated to 4 ml in a Kuderna-Danish evaporator. Lipids were then removed by HPLC using two 100 Å, 122 cm Styragel® columns with a methylene chloride flow rate of 4·0 ml/min. The sample was then analysed by GC as described above. Spiked fish samples gave recoveries of 94–105%.

Lipid analyses for each tank were carried out using fish samples containing between fifteen and thirty fish collected on days 5 and 12. The samples were Soxhlet extracted as described above and dried to constant weight.

*Statistics*

Fish weight data for each tank were derived from pooled fish samples collected daily for phenanthrene analysis during the 14-day flow-through exposure. The average fish weights for the two tanks were compared using the Wilcoxon matched-pairs signed-ranks test. In addition, the slopes of regression lines (fish weight *versus* time) were compared using the $t$ test.

The bioconcentration factor (BCF) and its error (E) on day $t$ were calculated as follows:

$$\mathrm{BCF}_t = \frac{F_t}{W_{0-t}}$$

$$\mathrm{E}_t = \sqrt{\left(\frac{\mathrm{E}F_t}{F_t}\right)^2 + \left(\frac{\mathrm{E}W_{0-t}}{W_{0-t}}\right)^2} \times \mathrm{BCF}_t$$

where: $F_t$ = phenanthrene concentration (μg/kg) in fish sample on day $t$; $W_{0-t}$ = phenanthrene concentration (μg/litre) in tank water, i.e. mean of all values from day 0 to day $t$; $\mathrm{E}F_t$ = analytical error in fish phenanthrene determination on day $t$; $\mathrm{E}W_{0-t}$ = standard deviation of phenanthrene concentration of tank water from day 0 to day $t$ and $\mathrm{E}_t$ = absolute error in $\mathrm{BCF}_t$ on day $t$.

## RESULTS AND DISCUSSION

Parameters measured during the coal exposure are shown in Figs 1 and 2. The particle size analysis (Fig. 1) indicated that 92 °$_o$ of the particles were 10 $\mu$m or less in the coal particulate tank on day 0. The variation among quadruplicate counts of this sample was 2–5 °$_o$ in the 1–30 $\mu$m size class and 11 °$_o$ in the 30–60 $\mu$m size class. In Fig. 2 the mean and standard deviation of the turbidity measurements taken during the 14-day test period were $0{\cdot}69 \pm 0{\cdot}47$ NTU (control tank, $n = 14$) and $3{\cdot}19 \pm 0{\cdot}80$ NTU (coal tank, $n = 14$). Particulate concentrations were $1{\cdot}94 \pm 0{\cdot}82$ mg/litre in the control tank ($n = 14$) and $10{\cdot}34 \pm 2{\cdot}90$ mg/litre in the coal tank ($n = 28$). The particulate concentrations in the control tank showed the contributions of food, faeces, etc. under these experimental conditions so it was estimated that around 80 °$_o$ of the suspended solids in the coal tank were coal particulates.

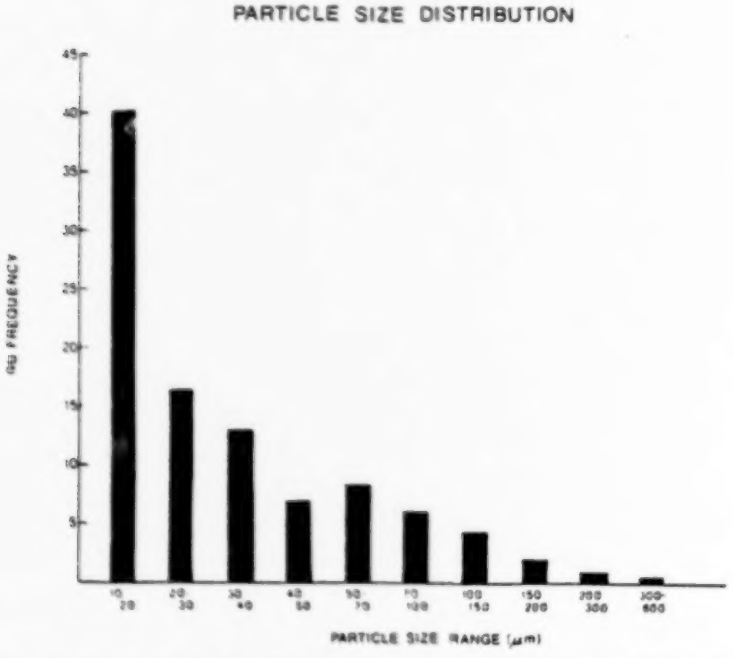



FIG. 1. Particle size distribution of coal particulate suspension just prior to the introduction of fish (day 0).

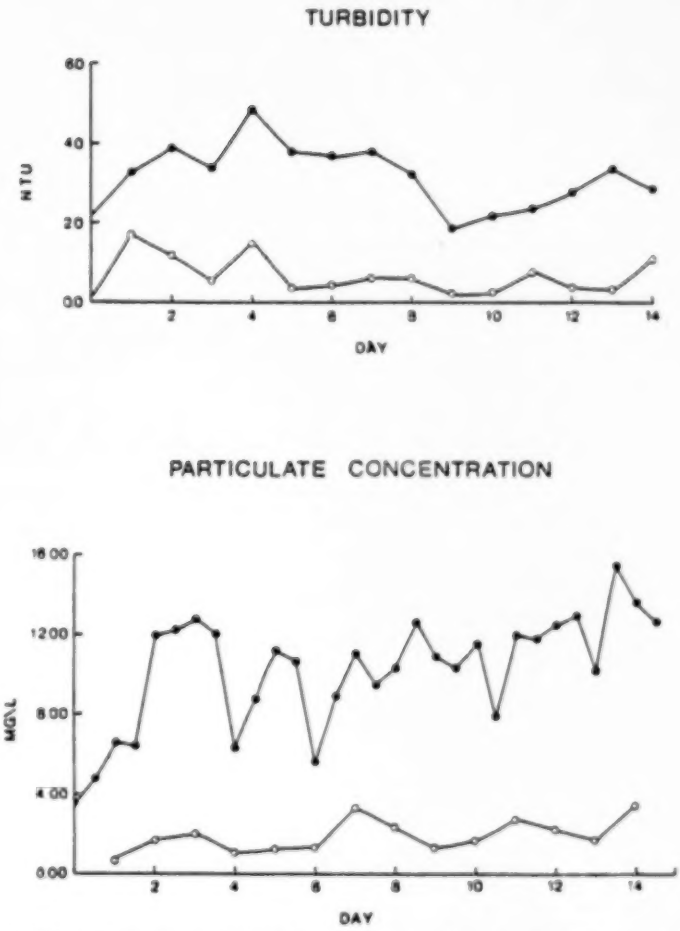



Fig. 2. Turbidity and particulate concentration measurements for coal tank (●) and control tank (○) during the 14-day experimental period.

*Histology*

Light microscopic observation of transverse sections from whole fish showed no obvious damage to external or internal epithelial surfaces by coal particulates. Careful examination of gill filaments showed neither morphological changes nor particulate entrapment in coal-exposed fish. We had previously observed clogging of gills at high concentrations (25,000 mg/litre) of coal particulates (Carlson *et al.*,

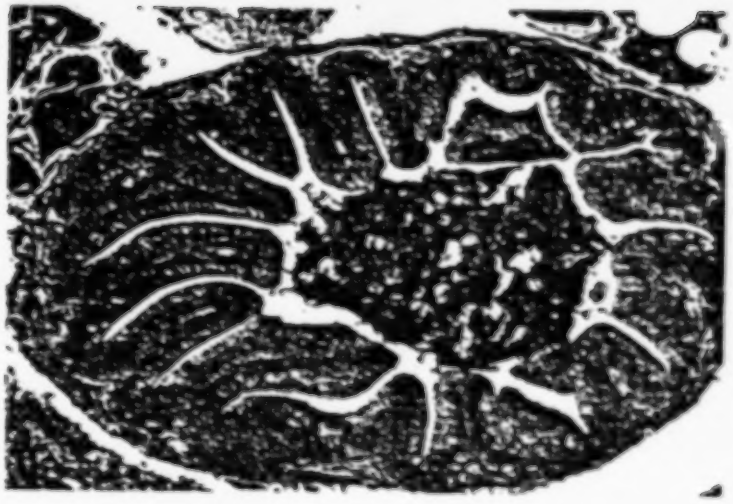
1979) and this phenomenon has also been reported during high sediment (Wallen, 1951) and ferric hydroxide (Coutant *et al.*, 1978) exposures.

However, a marked proliferation of mucous globules was observed in the gut of coal-exposed minnows. The photographs in Fig. 3 show numerous mucous globules present in the goblet cells of coal-exposed fish but not in control fish. This physiological response is consistent with the idea that mucous secretion is a mechanism for lubricating and protecting the gut from abrasion (Barnard, 1973). It is possible that villar absorption of food is hindered by extensive mucous generation and, if so, this response might contribute to the decline in growth rate observed in particulate-exposed animals. For example, lower weights were measured in rats fed a diet containing over 10 % asbestos fibres for a 6-week period (Donham *et al.*, 1980). In an aqueous exposure rainbow trout showed a depression of growth over a 10-month period in 50, 100 and 200 mg/litre solutions of coal-washery wastes. Similarly, Smith *et al.* (1973) noted a significant inhibition of growth in fathead minnows exposed to ferric hydroxide suspensions over a 5-month period which they attributed to impaired food perception and stress. If the fishes ingested particulates during these experiments, this also might have contributed to their lower growth rates. During the short time frame of our experiment no statistical difference in daily fish weight or in growth rate was found between tanks when pooled fish samples (14–30 fish per tank per day) were compared.

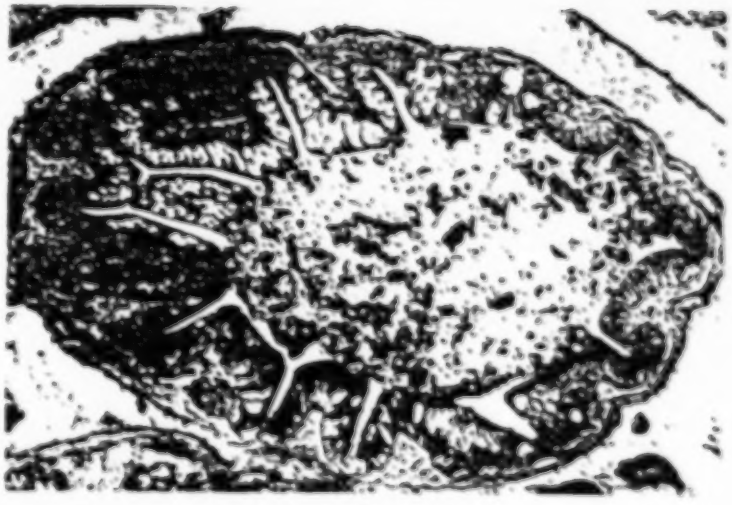
The appearance and disappearance of mucous globules reflected the rapid ingestion and elimination of coal particulates in the gut. Based on the static exposure, there was a 3 h lag-time between the first appearance of scattered coal particles (4 h) and the observation of a few mucous globules (7 h). Fish were first collected at 16 h during the flow-through exposure and at that time numerous particulates and mucous globules were seen. Following the 14-day exposure period, the coal was rapidly cleared from the gut with no observable coal particles present 24 h after the coal input ceased. At that time mucous globules were still seen but after 48 and 72 h the gut mucosa was indistinguishable from that of control fish. No differences between fish from the phenanthrene control tank and fish from Lake Superior water were observed.

Coal particles were not observed within the gill epithelium or gut mucosa. However, as discussed by Cook (1979), light or electron microscopic examination of histological sections is not a particularly sensitive method for detecting particulate penetration if particulates are present in low concentrations, if the particulates are not localised and if the particulates are small ($< 1\ \mu m$). Yet the transport of a variety of particulates, including carbon particles, into the body cavity has been documented microscopically in several mammalian studies (Volkheimer, 1974; LeFevre & Joel, 1977).

Electron microscopic examination of the gut showed no erosion of the microvilli or other signs of damage to the mucosa (Fig. 4). Predictably, numerous mucous formations were present, as shown in Fig. 4(b). In contrast, Jacobs *et al.* (1978)



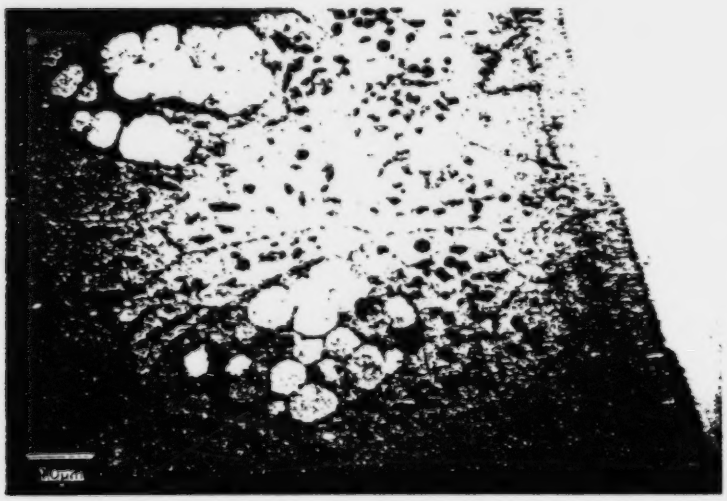
(a)



(b)

Fig. 3. Cross-sections of fathead minnow gut. The 3 μm sections were stained with methylene blue-azure II-basic fuchsin, X100. (a) Control fish with food in lumen, day 14. (b) Coal-exposed fish with coal particulates in lumen following a 14-day exposure period; note extensive mucous formation.

(a)



(b)

Fig. 4. Thin sections of fathead minnow gut X5000. (a) Control fish. (b) Coal-exposed fish with mucous globules.

described particulate-induced damage to the gut of rats fed chrysotile asbestos for 14 months. Light and electron microscopic examination of the ileum, where damage was particularly severe, showed erosion of the tips of the villi, loss of cellular tight junctions, loss of microvilli, abnormal blebbing of the microvilli and even destruction of some epithelial cells.

*Bioaccumulation*

The phenanthrene concentrations of tank water, fish and suspended particulates during the 14-day flow-through exposure are shown in Table 1. Fish samples

TABLE 1
PHENANTHRENE CONCENTRATIONS OF FISH, WATER AND PARTICULATES DURING 14-DAY FLOW-THROUGH EXPOSURE

| *Day* | *Control tank* | | *Coal tank* | | |
|---|---|---|---|---|---|
| | *Fish*[a] (μg/kg) | *Water* (μg/litre) | *Fish*[a] (μg/kg) | *Water* (μg/litre) | *Particulates*[b] (μg/litre) |
| 3 | 185 ± 30 | 0·082 ± 0·026 (n = 5) | 148 ± 75 | 0·088 ± 0·009 (n = 5) | 1·015 ± 0·429 (n = 4) |
| 10 | 243 ± 130 | 0·075 ± 0·022 (n = 15) | 305 ± 211 | 0·080 ± 0·022 (n = 15) | 0·938 ± 0·309 (n = 11) |
| 14 | 933 ± 55 | 0·076 ± 0·021 (n = 19) | 341 ± 66 | 0·084 ± 0·023 (n = 19) | 1·048 ± 0·350 (n = 15) |

[a] Pooled fish samples containing 17–20 fathead minnows (2–4 g wet weight) were used.
[b] The phenanthrene content of total suspended solids was determined and divided by the volume of tank water filtered.

collected on day 15—the first day of the depuration period—had no detectable phenanthrene in either control or coal-exposed fish. This is in agreement with our previous studies (Carlson *et al.*, 1979), where phenanthrene was rapidly eliminated by fathead minnows. Lipid analyses from this experiment gave values of 5·66 ± 0·69 % ($n = 2$) on day 5 and 8·17 ± 0·46 ($n = 2$) on day 12, which were above the range of our previously reported values, 3·8–4·8 %.

The fish and water data from Table 1 were used to calculate the BCFs shown in Table 2. For comparison, BCFs from our previous bioaccumulation experiments (Carlson *et al.*, 1979) were also included. Veith *et al.* (1979) have related log BCF to log $P$ (partition coefficient) in an empirically derived equation, $\log BCF = 0{\cdot}85 \log P - 0{\cdot}70$. Our values for phenanthrene bioconcentration fit this correlation reasonably well. Based on a log $P$ of 4·46 for phenanthrene (Leo *et al.*, 1971), the calculated log BCF is 3·09. The observed log BCFs were 3·35–4·09 in the phenanthrene-only tank and 3·23–3·61 in the phenanthrene plus coal tank.

BCF values for the coal-exposed fish were within the range of the other BCFs reported in Table 2, indicating water uptake as the major route of phenanthrene bioaccumulation. Comparison of coal with control tank values showed that the two were within experimental error on each day except on day 14 when the control BCF exceeded the coal BCF. The reason for the anomalously high BCF of 12,000 in the

control tank is not known. Based on the ratio of particulate phenanthrene to aqueous phenanthrene, BCF values of up to twelve times higher than controls might have been found in coal-exposed fish if particulates contributed significantly to aqueous uptake. However, using our experimental design, BCFs would have had to have increased at least two-fold to have been detected because of the error in the BCF determination and the variability among experiments. Therefore, small contributions by the coal particulates could not have been measured.

Coal particulates were ingested by the fish apparently during browsing and

TABLE 2
BCFs FOR FATHEAD MINNOWS EXPOSED TO SOLUBLE AND PARTICULATE-BOUND PHENANTHRENE

| *Exposure* | *Day* | | | |
|---|---|---|---|---|
| | 3 *or* 4 | 7 | 10 | 14 |
| Coal[a] | 1690 ± 870 | — | 3810 ± 2830 | 4060 ± 1360 |
| Control[a] | 2250 ± 800 | — | 3240 ± 1980 | 12440 ± 3560 |
| 2-1[b] | 800 ± 340 | 1280 ± 470 | 1530 ± 440 | — |
| 2-2[b] | 1870 ± 320 | 2260 ± 130 | 3340 ± 290 | — |
| 3-1[b] | 1580 ± 500 | 1760 ± 380 | — | 1900 ± 390 |
| 3-2[b] | 1530 ± 130 | 1460 ± 240 | — | 1310 ± 250 |
| 3-3[b] | 2350 ± 530 | 1960 ± 380 | — | 3040 ± 420 |

[a] Calculated from values in Table 1.
[b] Recalculated phenanthrene BCF values from Carlson *et al.* (1979). The mean phenanthrene concentration in the tank water ranged from 1·97 to 3·15 μg/litre.

feeding activities. As discussed above, numerous coal particulates were seen in the histological sections of the gut and, in addition, the faeces were noticeably black after 24 h and throughout the exposure. Therefore to determine the amount of phenanthrene in the gut, thirty fish from each tank were gutted on day 14. Phenanthrene was not detected in the control gut extracts but a significant amount was found in the coal-containing gut sample, 962 ± 392 μg/kg. The gut weight was 13% of the total fish weight but it contributed a calculated value of 35% to the total phenanthrene content of the whole fish extract on day 14. This was based on a value of 0·94 μg of phenanthrene in the gut sample (0·98 g) and 2·70 μg of calculated phenanthrene in the whole fish sample (7·90 g). The error in the 35% value was 45%. The BCF values reported in Table 2 were based on whole fish analyses and therefore the BCFs for coal-exposed fish may be overestimated by at least 35% due to the contribution of coal particulates in the gut. However, even if the BCFs from coal-exposed fish were adjusted, they would remain within the predicted range. Moreover, the possibility of overestimating these BCFs gives further support to the idea of direct aqueous accumulations as the dominant route of uptake with little, if any, contribution by coal particulates.

This finding is in agreement with those of Southworth *et al.* (1979), who found that direct uptake of acridine from water (98%) was the major route of uptake in contrast to uptake from sediment (1%) in fathead minnows. Also, McCain *et al.* (1978)

exposed English sole to oiled sediments containing a variety of alkane and aromatic compounds. Several hydrocarbons were accumulated but although phenanthrene was detectable in the sediments it was not found in fish tissues. This may reflect the low water solubility and stable binding capacity of phenanthrene to the particulates studied.

In summary, histological examination of coal-exposed fish revealed no obvious damage to epithelial surfaces, but numerous mucous globules appeared in the gut mucosa in response to the presence of coal particulates in the gut. Although the phenanthrene-coated coal particles were readily ingested, enhanced bioaccumulation of phenanthrene was not measurable. Phenanthrene desorption in the gut lumen, phenanthrene–coal–membrane interactions with the gut mucosa and coal penetration into internal tissues were three possible mechanisms for adding to the direct bioaccumulation of phenanthrene from water. These possibilities were not considered to be major routes of uptake because BCFs from coal exposed fish were 1000–4000 during the 14-day exposure period which was in the range of control values and of previous experimental values. The last mechanism was also not considered a major contribution because of the absence of histologically observable accumulations of particulates in internal tissues. However, our methods would not have detected small contributions since even the presence of numerous phenanthrene-containing coal particles in the gut did not appreciably increase the whole-fish BCF. This experiment demonstrated that, in the case of phenanthrene, a hydrophobic compound, and of coal, an organic particulate, phenanthrene bioaccumulation was not enhanced significantly during a short-term exposure. Other compound–particulate combinations might react quite differently in aquatic organisms.

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